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RESEARCH

GIP-induced vasodilation in human adipose tissue involves capillary recruitment

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) in combination with hyperinsulinemia increase blood flow and triglyceride clearance in subcutaneous abdominal adipose tissue in lean humans. The present experiments were performed to determine whether the increase involves capillary recruitment. Eight lean healthy volunteers were studied before and after 1 h infusion of GIP or saline during a hyperglycemic–hyperinsulinemic clamp, raising plasma glucose and insulin to postprandial levels. Subcutaneous abdominal adipose tissue blood flow (ATBF) was measured by the ¹³³Xenon clearance technique, and microvascular blood volume was determined by contrast-enhanced ultrasound imaging. During infusion of saline and the clamp, both ATBF (2.7 ± 0.5 mL/min 100 g/tissue) and microvascular blood volume remained unchanged throughout the experiments. During GIP infusion and the clamp, ATBF increased ~fourfold to 11.4 ± 1.9 mL/min 100 g/tissue, $P < 0.001$. Likewise, the contrast-enhanced ultrasound signal intensity, a measure of the microvascular blood volume, increased significantly 1 h after infusion of GIP and the clamp ($P = 0.003$), but not in the control experiments. In conclusion, the increase in ATBF during GIP infusion involves recruitment of capillaries in healthy lean subjects, which probably increases the interaction of circulating lipoproteins with lipoprotein lipase, thus promoting adipose tissue lipid uptake.

Key Words

- ▶ glucose dependent insulinotropic polypeptide
- ▶ adipose tissue
- ▶ blood flow
- ▶ ultrasonic imaging
- ▶ microcirculation

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Introduction

The regulation of subcutaneous adipose tissue blood flow (ATBF) is crucial in lipid homeostasis (1, 2). It has long been recognized that the ATBF is tightly coupled to adipose tissue metabolism (3, 4, 5, 6). In response to feeding, an increase in ATBF is seen in healthy subjects (4). GIP is involved in the increase of ATBF in healthy subjects (7, 8, 9). GIP is a polypeptide hormone that is secreted from the small-intestinal K cells in response to nutrient intake (10). GIP is well founded as an incretin hormone potentiating insulin release from β -cells in healthy humans (10, 11, 12).

In addition to its insulinotropic effects, we have previously shown that GIP infusion during physiological hyperglycemia and hyperinsulinemia increases ATBF and stimulates glucose uptake and triacylglycerol (TAG) deposition in adipose tissue in lean humans (7). In contrast, in these experiments neither GIP nor hyperinsulinemia *per se* had any effect on ATBF. Subsequent studies in lean healthy subjects demonstrated that insulin is essential for GIP to increase ATBF and adipose tissue TAG clearance (9). In obese humans with impaired glucose tolerance,

GIP infusion in combination with hyperglycemia and hyperinsulinemia did not induce any changes in ATBF and TAG uptake in adipose tissue (13). However, weight loss (mean weight loss: 7.5 kg) induced by caloric restriction in obese humans with impaired glucose tolerance, resulted in improved insulin sensitivity and partly restored the vasodilatory effect of GIP and insulin during otherwise comparable experimental conditions (8). Recently, we demonstrated that the circulatory and metabolic effects of GIP in adipose tissue can be blocked by a truncated form of human GIP, GIP (3-30)NH₂, which is a high-affinity competitive antagonist of the human GIP receptor (14). In addition, we have previously shown that in subcutaneous abdominal adipose tissue of healthy lean subjects, there is an increased capillary recruitment in response to an oral glucose load, as indicated by an increased microvascular blood volume (15). The recruitment of capillaries will expose a larger amount of the circulating triacylglycerol particles to the endothelial lipoprotein lipase (LPL), increasing the hydrolysis of these particles. This, in turn, will give rise to an enhanced free fatty uptake in adipose tissue. Thus, the aim of this study was to elucidate whether the effects of GIP on ATBF and lipid metabolism involves capillary recruitment in healthy lean subjects.

Materials and methods

Subjects

Eight healthy non-smoking males were studied. The anthropometric characteristics, subcutaneous adipose tissue thickness, body fat composition, lipid and glucose profile are summarized in Table 1. The subjects also took part in a recent, reported study (14). None of the subjects had a positive family history of diabetes. All had normal fasting blood concentrations of glucose,

lipids and hemoglobin and normal renal and hepatic functions. All subjects gave informed written consent to participate. The protocol was approved by the Ethical Committee of Copenhagen Municipality (H-2-2010-079), and the study was performed in accordance with the Helsinki Declaration II.

Experimental design

The study protocol had a cross-over design with a washout period of at least 2 weeks. Each subject served as his own control and was studied, in a randomized order, on two different occasions during infusion of GIP (1.5 pmol/kg/min) or saline during 60 min – both days in combination with physiological hyperglycemic-hyperinsulinemic (Hy_{gluc}-Hy_{ins}) clamps, raising plasma glucose and insulin to postprandial levels.

Protocol

The subjects were instructed to abstain from drinking alcohol or doing strenuous physical activities for 2 days prior to the experiments to ensure equally filled glycogen stores. On the days of the experiments, subjects arrived at the department at 08:00 h having used non-strenuous transportation and after having fasted for at least 12 h. The investigations were performed with the subjects in supine position in a room kept at 24°C. A catheter (BD Venflon, TMPRO, Becton Dickinson, Singapore) was inserted into an antecubital vein for the infusion of GIP, saline, glucose, insulin and SonoVue (see below).

After baseline measurements (time –30, –15 and 0 min) a continuous infusion of either GIP or saline in combination with the clamp were commenced.

GIP infusions and the clamp

GIP

Synthetic human GIP (1–42) was purchased from Bachem (Bubendorf, Switzerland) and demonstrated to be >97% pure and identical to the natural human peptide by HPLC, mass and sequence analysis. GIP was dissolved in saline containing 0.5% human serum albumin (Human Albumin, CSL Behring, Marburg, Germany) and subjected to sterile filtration.

Hy_{gluc}-Hy_{ins} clamp

The objective of the Hy_{gluc}-Hy_{ins} clamp was to increase plasma glucose levels above fasting levels (6.5–7 mM) and

Table 1 Anthropometric characteristic, lipid and glucose profile of studied male subjects.

Characteristics	Mean ± s.d.
Age (years)	26.4 ± 2.3
Height (cm)	182.4 ± 5.1
Body weight (kg)	76.5 ± 7.6
BMI (kg/m ²)	22.9 ± 1.4
Subcutaneous fat (cm)	2.6 ± 0.3
Fat mass (%)	18.9 ± 4.3
Triacylglycerides (mM)	1.0 ± 0.5
HDL (mM)	1.5 ± 0.2
LDL (mM)	1.9 ± 0.4
Cholesterol (mM)	4.0 ± 0.4
HbA1c (mmol/mol)	31 ± 1.4

to increase insulin concentration to postprandial levels. The protocol included: (1) a continuous, variable glucose infusion (the infusion rate was adjusted every 5–10 min according to measured plasma glucose to maintain the glucose concentration at the desired target); and (2) during saline infusion, insulin (Actrapid Human; Novo Nordisk) was infused at a continuous rate of 10 mU/m²/min. During concomitant GIP infusion, insulin infusion rate was adjusted according to the expected endogenous insulin secretion in response to GIP aiming toward plasma insulin levels comparable to those obtained during insulin infusion alone. Therefore, insulin was infused at a lower rate 7 mU/m²/min under these conditions.

Contrast-enhanced ultrasound imaging and image analysis

Contrast-enhanced ultrasound imaging has previously been validated in our laboratory and is a reliable method to estimate the microvascular blood volume in human subcutaneous abdominal adipose tissue (15). An ultrasound transducer was placed vertically and each time at the same side of the abdomen approximately 5 cm above the iliac crest and at the para-umbilical area in the mid-clavicular line. The location of the transducer was highlighted before the infusions were started; thus, it was possible to use the same location later (1 h after the start of infusions). The transducer was held in a fixed position and the subjects were instructed not to change body position during the measurements. At baseline and 1 h after GIP or saline infusion, a bolus of 2.5 mL of the ultrasound contrast agent (SonoVue Bracco S.p.A, Milan, Italy) was injected intravenously followed by an immediate flush of 10 mL 0.9% saline solution. SonoVue is a suspension of phospholipid-stabilized microbubbles filled with hexafluoride; it is diluted with 4.5 mL 0.9% saline solution before injection (8 μ L microbubbles/mL). Ultrasound scanning was performed using a linear array transducer (L9-3 MHz) and an iU22 ultrasound scanner (Phillips Medical Systems, Bothell, WA, USA). B-mode imaging is created from broadband spectra of acoustic frequencies from 3 to 9 MHz. Contrast first harmonic signals were received at 8 MHz with mechanical index of 0.06. The depth was set at 3 cm allowing for measurements in subcutaneous abdominal adipose tissue. Focus and gain were optimized at the beginning of the experiment and held constant throughout. Twenty millisecond images were captured consecutively for 2 min after each bolus injection.

Image analysis was performed off-line using an ultrasound quantification and analysis software (QLAB version 7; Phillips Medical Systems). The analysis consisted of examination of region of interest (ROI) for each recording in the subcutaneous abdominal adipose tissue. The ROI placements were in areas that excluded larger vessels during the enhancement phase. In a ROI, the relative microvascular blood volume expressed in Δ decibels (dB) in the tissue was determined as the mean signal intensity in dB during the first plateau phase after subtraction of the mean signal intensity registered immediately before the contrast bolus injection, Fig. 1 (16).

Determination of ATBF

ATBF in the subcutaneous abdominal adipose tissue contralateral to the ultrasound transducer position was determined during the whole experiment by recording washout of ¹³³Xenon, which was injected in gaseous form into the adipose tissue. This technique has previously been validated in our laboratory (17). About 1 MBq gaseous ¹³³Xenon mixed in about 0.1 mL of atmospheric air was injected into the para-umbilical area of the subcutaneous abdominal adipose tissue. The washout rate of ¹³³Xenon was measured continuously by a scintillation counting device placed over the exact site of injection and fixed firmly in place (Mediscint, Oakfield Instruments, Oxford, UK). The ATBF was calculated from the mean washout rate constant determined in 30 min periods corresponding to the time points when blood samples were drawn. A tissue/blood partition coefficient for ¹³³Xenon of 10 mL/g was used. In order to detect early flow changes, a monoexponential curve was fitted to the washout curve registered in the basal control period

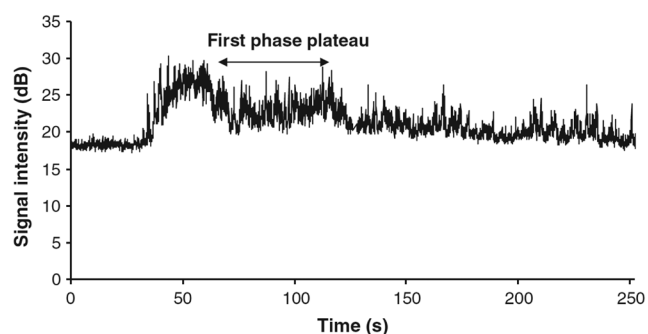


Figure 1

A curve from the subcutaneous abdominal adipose tissue after administration of a bolus of the ultrasound agent SonoVue® in one subject. The first-phase plateau level is used for the calculation of the microvascular blood volume.

immediately before the hormone infusions were initiated. The commencement of the vascular effects was defined when the slope of the $^{133}\text{Xenon}$ -washout curve deviated from the baseline slope by visual inspection.

Blood samples

Arterial blood samples were drawn at times -30 , -15 , 0 , 30 and 60 min. Blood samples were collected in iced chilled tubes (Vacuette; Greiner Labortechnik, Kremsmünster, Austria) from the artery for measurements of GIP and insulin. Tubes for total GIP contained EDTA and tubes for insulin contained heparin. All samples were centrifuged within 15 min for 15 min at $5000g$ at 4°C and stored at -80°C until analysis.

Blood analysis

Total plasma GIP concentrations were measured using a C-terminally directed antibody (18). Plasma insulin concentrations were measured by auto-DELPHIA automatic fluoroimmunoassay (14). Plasma glucose concentrations were measured bedside during the clamp experiments, using the Radiometer ABL 725 blood gas analyzer (Radiometer Corp., Brønshøj, Denmark).

Calculations and statistical analysis

The ATBF was calculated from the mean washout rate constant determined in 30-min periods corresponding to the time points when blood samples were drawn. ATBF was then calculated according to the equation $\text{ATBF} = -k \times \lambda \times 100$, where k is the washout constant and λ is the tissue/blood partition coefficient (λ) for $^{133}\text{Xenon}$.

Sample size was determined before the study using power analysis ($P=0.8$, $\alpha=0.05$) with regard to ATBF responses based on our previous publications (7, 8, 9, 13, 14). Assuming an ATBF response as found previously in normal weight, healthy subjects, less than six subjects were needed in the present study.

All results are presented as $\text{mean} \pm \text{s.e.m.}$ The significance of changes in ATBF with time was evaluated using two-way ANOVA for repeated measures. Changes in ultrasound signal intensity from baseline during GIP and saline infusions were compared, using paired t test (two-tailed). P values of <0.05 were considered statistically significant. The Spearman correlation test was used to calculate the correlation between ATBF and microvascular blood volume.

Data were analyzed using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Arterial glucose, insulin and GIP concentrations

Figure 2 shows plasma glucose, insulin and total GIP concentrations during GIP and saline. The concentrations of glucose, insulin, GIP during infusion of GIP have been a part of our recently published study (14), however, in the present study, besides baseline concentrations, only the concentrations during the first hour after commencement of GIP infusion and the clamps are shown and described, corresponding to the time capillary recruitment was measured. The clamps were carried out successfully. Plasma glucose levels reached the target levels of 6.8 ± 0.1 and 6.9 ± 0.1 mM 30 min after start of the infusion of GIP and saline, respectively, Fig. 2A. There was no significant difference between the amounts of glucose infused during these two experiments (27 ± 5 and 17 ± 3 g, $P=0.2$), reflecting similar insulin levels during the two experiments. Plasma insulin levels increased significantly and reached levels of 404 ± 8 and 358 ± 15 pM 30 min after the start of infusion of GIP and saline, respectively, Fig. 2B, $P=0.1$.

During GIP infusion, total plasma GIP concentrations increased significantly from fasting levels (9.9 ± 0.3 pM) and after approximately 30 min reached mean plateau levels of 120 ± 7 pM, whereas plasma GIP levels remained unchanged and at fasting levels during saline infusion (8.1 ± 0.9 pM), (Fig. 2C).

Changes in subcutaneous abdominal ATBF

Figure 3 shows the subcutaneous abdominal ATBF during the two experiments. Baseline ATBF did not differ between the two studies (2.6 ± 0.6 and 2.5 ± 0.5 mL/100g/min). ATBF during GIP infusion has been described in our recent study (14) but in the present study only ATBF during the first hour after commencement of infusion of GIP and the clamps are shown and described. During GIP infusion ATBF increased fourfold to a mean level of 11.4 ± 1.9 mL/100g/min ($P=0.004$), whereas ATBF remained unchanged during saline infusion (2.7 ± 0.5 mL/100g/min).

Changes in microvascular blood volume

During the two experiments, baseline ΔdB signal intensities were almost similar (2.1 ± 0.3 and 1.5 ± 0.3 dB,

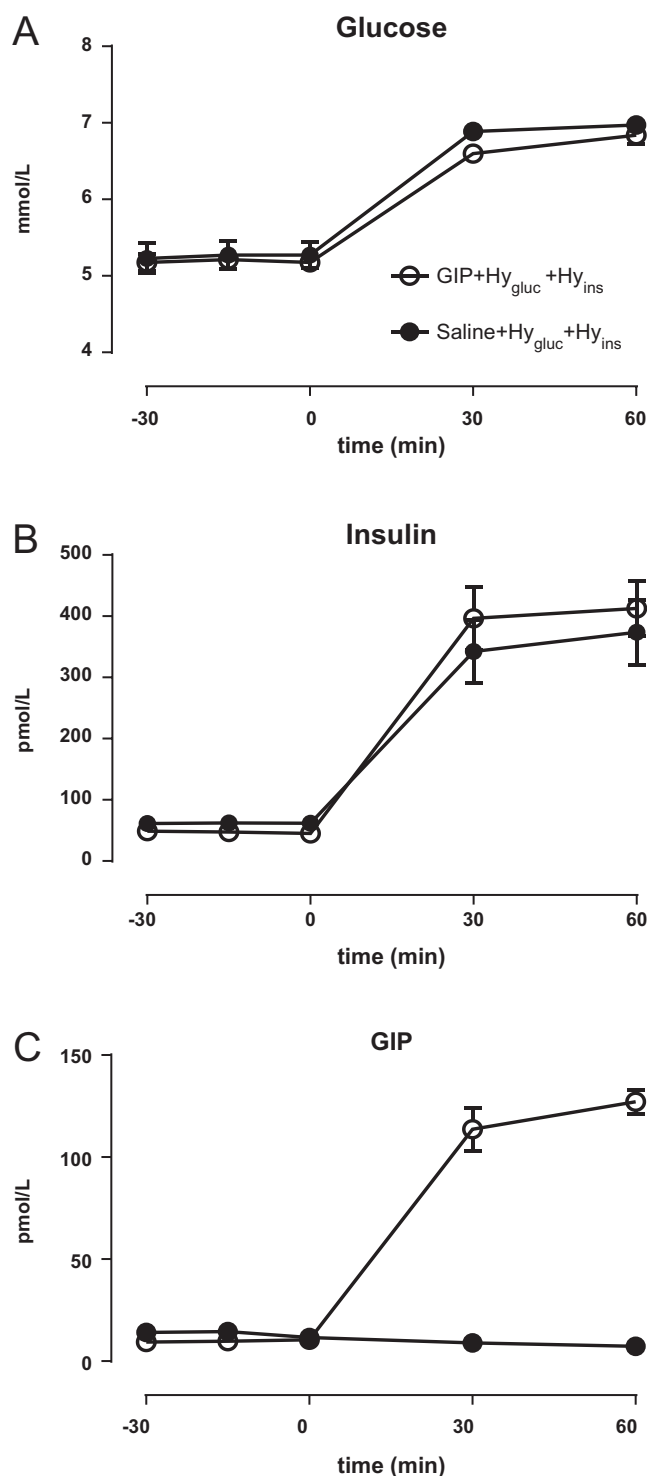


Figure 2
Plasma glucose (A), insulin (B) and GIP (C) concentrations during Hy_{gluc}-Hy_{ins} clamp with GIP infusion (open circle) and saline (solid circle). Data are mean \pm S.E.M.

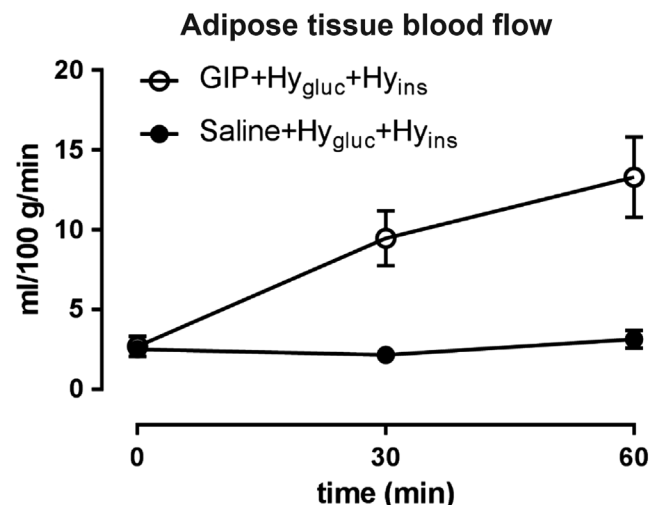


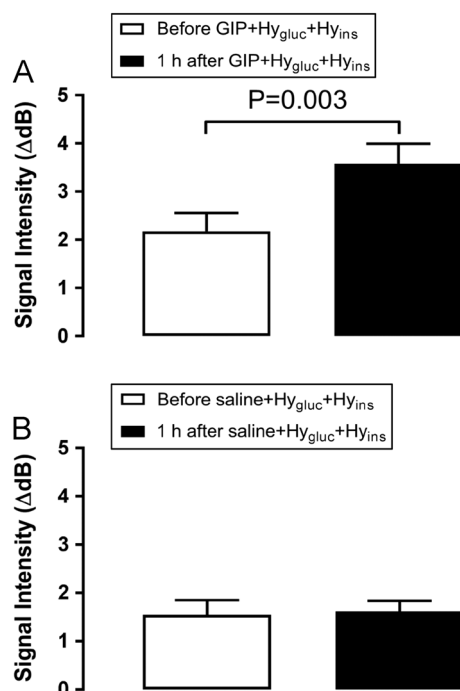
Figure 3
Subcutaneous abdominal adipose tissue blood flow during Hy_{gluc}-Hy_{ins} clamp with GIP infusion (open circle) or saline (solid circle). Data are mean \pm S.E.M.

respectively, $P=NS$, Fig. 4A and B). After 60 min of GIP infusion, the ΔdB signal intensity increased significantly ($3.6 \pm 0.4 dB$, Fig. 4A, $P=0.003$), whereas no change was seen in the saline study ($1.6 \pm 0.2 dB$, Fig. 4B).

There was a significant correlation between microvascular blood volume and ATBF during GIP ($r=0.85$ and $P=0.01$). No correlation was seen during saline ($r=0.34$ and $P=0.5$).

Discussion

ATBF has a fundamental role in the regulation of the adipose tissue metabolism (4). We have previously shown in several studies that infusion of GIP in combination with insulin increase ATBF up to fivefold (7, 9, 14). In line with these findings we now show that the increase in ATBF during GIP and insulin plasma levels identical to those reached postprandially involves capillary recruitment. The phenomenon, capillary recruitment, has long been recognized in the skeletal muscle (19, 20, 21). Few studies have suggested that adipose tissue possesses the ability of capillary recruitment (15, 22). However, in the postprandial period, adipose tissue metabolism is, at least in part, dependent on increase in the macrovascular and microvascular perfusion rate (23). The increase in blood flow in adipose tissue following feeding parallels that of plasma GIP and insulin concentrations (24).

**Figure 4**

Signal intensity changes from baseline to first-phase plateau (Δ dB) in subcutaneous abdominal adipose tissue before and 1 h after Hy_{gluc}-Hy_{ins} clamp with (A) GIP and (B) saline.

Several mechanisms influence the regulation of the postprandial ATBF in humans (23). In humans, adrenergic mechanisms are dominating, with β_1 -receptor-mediated vasodilatation (3, 6) and α_2 -receptor-mediated vasoconstriction being the main regulators (25). The blood flow response to feeding is blocked by propranolol infusion (completely in some adipose tissue depots and partially in others) (6), suggesting that it is dependent upon sympathetic activation. The mechanisms behind the induction of the capillary recruitment in the adipose tissues are yet unknown, but a study from our laboratory showed that infusion of adrenaline increased both ATBF and capillary recruitment (26).

In human and animal studies, insulin exerts vascular actions in skeletal muscle, increasing blood flow and glucose disposal (27, 28). It recruits microvasculature in the muscle by relaxing terminal arterioles thereby expanding the microvascular blood flow (20, 21, 29). Furthermore, insulin induces relaxation of resistance vessels and causes an increase of overall blood flow (27, 30). The insulin-induced microvascular recruitment in muscle is mainly mediated by nitric oxide (NO) (31). NO exerts a major role in vascular biology by regulating vascular tone and

mediating vasodilation (32). The same pathway may also be relevant for GIP's action in the adipose tissue microvasculature, as the GIP-induced vasodilatory actions appear to be mediated via endothelium-dependent NO production (33). In the present study hyperinsulinemia *per se* did not have any effect on neither the ATBF nor the adipose tissue microvascular blood volume. These findings are in accordance with our previous studies in which we found that neither insulin nor GIP *per se* had any effect on ATBF but that GIP in the presence of insulin above a certain concentration increased ATBF and adipose tissue TAG deposition (7). Administration of even larger amounts of insulin did not elicit further increase in ATBF indicating that insulin exerts a permissive but not additive role for the vasodilatory effect of GIP (9). This effect of GIP is most likely receptor mediated as it can be abolished when antagonizing the human GIP receptor (14). GIP receptors are found also in human adipose tissue (34) and recent studies indicate that GIP receptor expression in subcutaneous adipose tissue is inversely associated with BMI (35, 36).

Interestingly, the other incretin hormone, glucagon-like-peptide-1 (GLP-1), also increases total blood flow in adipose tissue in healthy subjects, however, with a delay compared to the effect of GIP (37). Furthermore, GLP-1 has been shown to increase microvascular recruitment in human and rat muscle (38). Whether GLP-1 also increases microvascular recruitment in adipose tissue remains to be elucidated.

In the postprandial state, the adipose tissue's metabolic role is primarily to deposit circulating triglycerides (1). As the enzyme LPL is bound to the adipose tissue capillaries, the capillary recruitment, that is enlarged vascular surface area in the adipose tissue will increase the exposure of the circulating lipoproteins to the enzyme and lead to a postprandial increased lipid deposition in the adipose tissue, at least in healthy subjects (1). This coupled with our previous findings (7, 9, 14), where we found an increase in TAG hydrolysis, FFA re-esterification and glucose uptake, is consistent with a coordinated GIP response in adipose tissue resulting in increased TAG deposition in subcutaneous abdominal adipose tissue via the conversion of glucose to alpha-glycerol-phosphate to esterify the free fatty acids derived from the TAG hydrolysis.

In conclusion, subcutaneous adipose tissue capillaries are recruited simultaneously with increased ATBF in response to GIP with insulin in healthy lean subjects, thus increasing the exposure of LPL to its substrate.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M A designed the study, recruited the subjects, supervised the studies, collected and analyzed the data and wrote the manuscript. A A recruited the subjects, supervised the studies, collected and analyzed the data and reviewed and edited the manuscript. L S supervised the studies, reviewed and edited the manuscript. F D analyzed the samples and reviewed the manuscript. J J H analyzed the samples and reviewed the manuscript. J B designed the study, supervised the studies, reviewed and edited the manuscript.

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